

Investigation of Antidiarrheal Activity of the Aqueous Leave Extract of *Ficus asperifolia* in Rats

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Abstract

Ficus asperifolia (FA) has been recommended locally for several disorders including diarrhea. Although various work had been done on *Ficus asperifolia* but not on its antidiarrhea activity. The objective of this research is to investigate the antidiarrheal activity of the aqueous leave extract of *Ficus asperifolia* in rats. This is to substantiate the traditional claim of the antidiarrheal activity of FA. The effect of aqueous leave extract of *Ficus asperifolia* on castor oil-induced diarrhea, castor oil-induced enteropooling, and gastrointestinal motility test using activated charcoal meal method were examined. The extract was first assayed for its effects in castor oil-induced diarrhea at different doses (400, and 800mg/kg, p.o.) in which significant activity ($p < 0.05$) was observed at a dose of 800mg/kg. Hence, the dose was used as the working dose in the other models. In induced Enteropooling and Intestinal motility, there was a significant reduction in secretion and distance travel by marker respectively. The result shows that aqueous leave extract of FA has an antidiarrheal activity by inhibiting secretion and intestinal motility.

Keywords: Aqueous extract; Antidiarrhea; Oil-induced; Enteropooling; Receptor blockades

Abbreviations: ORT: Oral Rehydration Therapy; PCV: Packed Cells Volume; AQFA: Aqueous Extract of *Ficus asperifolia*.

Introduction

Diarrhea accounts for more than 5 million deaths of children under five, especially in developing countries [1]

and despite the undeniable success of Oral Rehydration Therapy (ORT), acute diarrhea remains a leading cause of childhood deaths [1,2]. The greatest number of deaths from diarrhea occurs in South Asia and Africa and the disease is worsened by lack of access to clean water, malnutrition and hygienic disposal of human waste [3].

The use of medicinal plants is an age-long practice in various parts of the world for both curative and preventive measures [4]. Recently, there has been a great interest in herbal remedies for the treatment of a number of ailments including diarrhea [5] and in many African countries, herbal drugs are used in treating diarrhea especially in rural areas. Today it is estimated that about 80% of the world population rely on plant extracts as medicine to meet their health needs [4]. According to the statement of World Health Organization (WHO), medicinal plants would be the best source from which to develop a variety of medications for diarrhea [1].

Ficus asperifolia has the traditional claim of having antidiarrheal activity, but the potency and efficacy has not been scientifically determined. This research work was carried out to evaluate the antidiarrheal activity of aqueous extract of *Ficus asperifolia* (AQFA) in order to prove the traditional claim scientifically. *Ficus asperifolia* is a small size plant belonging to the family Moraceae. It is an epiphyte and a terrestrial plant which can reach a height of 20m. It is widely distributed in Nigeria, Senegal, Tanzania, Chad, Guinea-Bissau, Sierra Leone and many other African countries. The leaves are enormous and displayed spirally, the branches have the shape of ellipse and the roots are most of *Ficus asperifolia* is a highly medicinal plant that has been used in traditional medicine for the treatment of diseases, such as wound and diabetes [6]. Literatures reveal that *Ficus asperifolia* possess many pharmacological and physiological activities [7]. Recent studies reveal that oral LD₅₀ of aqueous leaf extract of *Ficus asperifolia* is higher than 4000mg/kg b. wt. Therefore, *Ficus asperifolia* can be categorized as highly safe since substances having LD₅₀ higher than 50mg/kg are non-toxic [4]. It was recently reported, that administration of aqueous extract of *Ficus asperifolia* caused a significant decrease in blood glucose concentration of alloxan-induced diabetic rats to normal levels [8,6]. It was also reported that aqueous extract of *Ficus asperifolia* have pro-implantation, pro-development, uterotrophic, and uterotonic-like activities [9]. Administration of *Ficus asperifolia* extract to rat with phenylhydrazine-induced anemia show increase in PCV (packed cells volume), Hb(hemoglobin),and RBC(red blood cell). All these show that *Ficus asperifolia* aqueous extract can be used to manage anemic patient [8].

This study was carried out to investigate the anti-diarrheal activity of aqueous extract of *Ficus asperifolia* (AQFA) leaf extract in experimental animal model.

Materials and Methods

Chemicals, Reagents and Drug

Castor oil, Normal saline, Loperamide, Atropine sulphate, Propranolol, Prazosin, Nifedipine, was purchased from registered pharmacy Negro Pharmacy, Anyigba, Kogi State Nigeria. While Tween 80, solvents and other chemicals were purchased from Sigma Chemical Co, Ltd (St. Louis USA). All of the chemicals and reagents were of analytical grade.

Plant material

The fresh leaves of *Ficus asperifolia* were collected from Egume and Kogi State University Anyigba campus in Dekina Local Government, Kogi State, Nigeria. The plant was identified and authenticated by a Taxonomist, in the Department of Biology, Faculty of Natural Sciences, Kogi State University, Anyigba, Nigeria. The sample was kept in the laboratory prior to the use.

Animals

Healthy Wistar albino rats of either sex weighing 100-150g were obtained from Animal House, College of Health Sciences, Kogi State University Anyigba, Nigeria.

Plant preparation and Extraction

The fresh leaves of *Ficus asperifolia* were washed with distilled water, and air dried in the laboratory at room temperature for four weeks. The plant sample was pulverized with mortar and pestle. A fine powder was obtained using a mortised blender and sieving (of pore). The fine powder was then stored under dried condition prior to use.

Exactly 200g of *Ficus asperifolia* leaves powder was weighed with the aid of analytical weighing balance and poured into a beaker containing 200ml of boiling water. The mixture was stirred with stirring rod, covered with a foil paper. After 24 hours, vacuum filtration was carried out using vacuum pump. The filtrate in a beaker was evaporated in a beaker in water bath at 100°C. The concentrated extract was kept in the refrigerator before use.

Experimental Design

A total of 40 albino Wistar rats were used for this research. The animals were housed under standard

environmental conditions (room temperature $26 \pm 1^\circ\text{C}$ and relative humidity 45 – 55%), with 12hours light/dark cycle. They were kept in cages, fed with pelletized feed (vital feed) and allowed free access to water throughout the research. The rats were allowed to acclimatize for two week before the experiments.

The experimental cages were lined with white blotting paper and wire gauze was placed about 2cm above the floor. For all the experiments, the animals were fasted for 18hours with free access to water.

Castor Oil Induced Diarrhea

The anti-diarrheal activity of aqueous extract of *Ficus asperifolia* (AQFA) was evaluated according to the method described by Teke, et al. [10]. Twenty (20) albino rats of either sex weighing 100 – 150g were used. They were divided into four groups of 5 rats each. The rats were fasted 18hours with free access to water before the experiment. All treatment in this experiment was through the oral route except otherwise stated. Each animal was administered 1ml of castor oil. Thirty minutes later, animals in the different groups were treated as follows: Group1 was given 10ml/kg normal saline; Group2, Loperamide (3mg/kg); Group3, AQFA (400mg/kg); Group4, AQFA (800mg/kg). The fecal pellets of the rats were thereafter counted every hour from the time of AQFA treatment for the first 5hours. The presence of wet faeces was noted for each animal. The dose with greater effect on wet fecal pellet output was used as the working dose.

Group	Number of rats	Description	Treatment
1	5	Induced with diarrhea	10ml/kg normal saline
2	5	Induced with diarrhea	3mg/kg Loperamide
3	5	Induced with diarrhea	400mg/kg AQFA
4	5	Induced with diarrhea	800mg/kg AQFA

Table 1: Showing the treatment of the castor oil induced diarrhea.

Intestinal Motility Test

Twenty albino Wistar rats were fasted for 18hours and divided into four groups of 5 animals each. Group 1 rats served as control and was administered 3ml/kg normal saline (p.o) orally. Group 2 served as positive

control, and received atropine sulfate (i.p) intraperitoneally. Group 3 and 4 received the aqueous extract of *Ficus asperifolia* (AQFA) (400 and 800mg/kg respectively) orally. After thirty minutes, all the animals received 1ml of activated charcoal (10% charcoal in Tween 80) orally. Thirty minutes later, the animals were sacrificed by cervical dislocation. The abdomen was cut open immediately and the whole small intestine (from pylorus to caecum) was dissected out. The length of the whole small intestine and the distance travelled by the marker (activated charcoal) was measured. This distance was expressed as a percentage of the length of the small intestine [3].

$$\text{Inhibition(\%)} = \frac{D_{\text{control}} - D_{\text{treated}}}{D_{\text{control}}} \times 100$$

D_{control} = distance travelled in control group

D_{treated} = distance travelled in treated group

Group	Number of rats	Marker	Treatment
1	5	10% charcoal in Tween 80	3ml/kg Normal saline
2	5	10% charcoal in Tween 80	0.1mg/kg Atropine
3	5	10% charcoal in Tween 80	400mg/kg AQFA
4	5	10% charcoal in Tween 80	800mg/kg AQFA

Table 2: Showing the summary of the intestinal motility test.

Castor oil - induced Enteropooling

Twenty albino Wistar rats of either sex were used for this experiment. They were fasted for 18hours and grouped into four groups of 5 animals each. Animals in group 1 served as control and received orally 1ml/kg of normal saline. Group 2 served as standard and received 3mg/kg Loperamide, while group 3 and 4 received AQFA (400mg/kg and 800mg/kg respectively) orally. One hour later, all the animals were administered 1ml of castor oil orally. After one hour of administration of castor oil, all the rats were sacrificed by cervical dislocation. The abdomen was cut open immediately and the whole of the small intestine was removed and weighed. The fluid content of the intestine was collected and measured. The intestine was re-weighed after collecting the fluid content, and the difference between the full and small intestine was calculated [3].

$$\text{Inhibition}(\%) = \frac{W_{\text{control}} - W_{\text{treated}}}{W_{\text{control}}} \times 100$$

W_{control} = weight of intestinal content in control group

W_{treated} = weight of intestinal content in treated group

Group	Number of rats	Pre-treatment	Treatment
1	5	1ml normal saline	1ml of castor oil
2	5	3mg/kg Loperamide	1ml of castor oil
3	5	400mg/kg AQFA	1ml of castor oil
4	5	800mg/kg AQFA	1ml of castor oil

Table 3: Show the summary of castor oil - induced Enteropooling.

Statistical Analysis

The results are presented as mean \pm standard error of mean. T-test was used to calculate the significant

Group	Treatment	1hour	2hours	3hours	4hours	5hours	Inhibition (%)
1	Control (NS)	2.4 \pm 0.22	2.4 \pm 0.22	2.4 \pm 0.22	2.6 \pm 0.24	3.6 \pm 0.36	-
2	Loperamide 3mg/kg	1.0 \pm 0.2 ^a	1.4 \pm 0.22 ^b	1.4 \pm 0.2 ^b	1.4 \pm 0.22 ^b	1.4 \pm 0.22 ^b	61.11
3	AQFA 400mg/kg	2.4 \pm 0.54	2.4 \pm 0.54	2.4 \pm 0.54	2.4 \pm 0.54	3.4 \pm 0.54	5.56
4	AQFA 800mg/kg	1.0 \pm 0.4 ^b	1.0 \pm 0.40 ^b	1.2 \pm 0.3 ^b	1.6 \pm 0.46 ^b	2.2 \pm 0.33 ^b	38.89

Table 4: Effects of AQFA on wet Fecal pellet output in castor oil-induced diarrhea. N = 5, b= significant compared with control at P < 0.05.

Effects of AQFA on Gastrointestinal Motility

Gastrointestinal motility test revealed that charcoal meal had moved 73.38 \pm 5.18% of the total length of the intestine in animal treated with normal saline after 30 minutes of ingesting the meal. Furthermore, the standard drug atropine significantly (p < 0.05) reduced the distance

differences between two groups. The values were considered significant when p < 0.05.

Results

Effects of Aqueous Extract of *Ficus asperifolia* on Wet Fecal Pellet Output

The result shows that administration of castor oil induced diarrhea in all the animals and treatment with aqueous extract of *Ficus asperifolia* at a dose of 800mg/kg significantly (p < 0.05) decrease the wet fecal pellet output within the first 5hours after diarrhea was induced using castor oil. The standard drug loperamide (3mg/kg) also significantly (p < 0.05) decreased the number of wet fecal pellet with an inhibition of 61.1% after 5 hours while the inhibition effected by 800mg/kg AQFA was 38.9% as shown in Table 1.

travelled by charcoal meal to 37.76 \pm 4.17%, while treatment with AQFA also reduced distance traveled by charcoal meal, with 400mg of the extract significantly (p < 0.05) reducing motility to 53.24 \pm 1.92, while 800mg/kg significantly (p < 0.05) reduced motility to 54.72 \pm 2.57 (Table 5).

Group	Total length of intestine (cm)	Distance travelled by marker (cm)	Percentage of intestinal motility (%)	Inhibition rate (%)
NS (3mg/kg) + CM	96.26	70.52	73.38 \pm 5.18	-
Atropine(0.1mg/kg) + CM	107.38	39.84	37.76 \pm 4.17*	43.51
AQFA (400mg/kg) + CM	102.92	54.88	53.24 \pm 1.92*	22.18
AQFA (800mg/kg) + CM	96.86	53.00	54.72 \pm 2.57*	24.84

Table 5: Effects of AQFA on Gastrointestinal motility.

Results were expressed as mean \pm standard error of mean; N= 5; * = significant compared with control group at p < 0.05. AQFA: aqueous extract of *Ficus asperifolia*. CM: charcoal meal. NS: normal saline.

Effects of AQFA on Castor oil-induced Enteropooling

Result shows that treatment with both doses of AQFA significantly ($p < 0.05$) reduced the weight of intestinal content, with 400mg/kg dose producing 32.61%

inhibition of intestinal fluid accumulation. Treatment with 800mg/kg effected 43.04% inhibition, while the standard drug loperamide produced 50.43% inhibition of fluid accumulation. The reduction in volume of intestinal content is in line with the weight as shown in Table 6.

Group	Weight of intestinal content (g)	Inhibition rate in volume of intestinal content (%)	Volume of intestinal content (ml)
NS (3ml/kg)	2.30	-	1.54 ±0.17
Loperamide (3mg/kg)	1.14*	50.43	0.6±0.11*
AQFA (400mg/kg)	1.55*	32.61	1.06±0.13 *
AQFA (800mg/kg)	1.31*	43.04	0.74±0.12*

Table 6: Showing the effects of AQFA and loperamide on intestinal fluid accumulation.

Results were expressed as mean ± standard error of mean; N= 5, * = significant compared with NS treated group at $p < 0.05$. AQFA: Aqueous extract of *Ficus asperifolia*. NS: normal saline.

Discussion

Diarrhea accounts for millions of deaths in the world annually. Most people who die from diarrhea actually die from severe dehydration and fluid loss. Therefore, the treatment of diarrhea is an important medical topic [3].

Castor oil is often used to induce diarrhea in animal model research, because castor oil is rich in ricinoleic acid, which causes irritation and inflammation of the intestinal mucosa [11]; stimulate the release of endogenous prostaglandins in the intestine, which in turn cause arachidonic-induced diarrhea, characterized by an increase in intestinal transit time and increase wet fecal pellet output [3].

Aqueous extract of *Ficus asperifolia* leaves (AQFA), which has not been studied so far for its anti-diarrhea activity was used in the treatment of diarrhea; castor oil induced enteropooling and intestinal motility tests were carried out. The result of this research reveals the anti-diarrheal activity of AQFA in experimental animal model. The study shows that AQFA treatment reduced fluid secretion in intestine and intestinal transit time. AQFA was not very effective at a dose of 400mg/kg but more effective at a higher dose of 800mg/kg.

Phytochemical screening of the plant extract revealed the presence of tannins, saponins, steroids, alkaloids which might be responsible for its medicinal/ anti-diarrheal activities. In addition, its anti-diarrheal action may be due to the presence of denatured proteins, which form protein tannates. It has been previously demonstrated that protein tannates make the intestinal

mucosa more resistant and hence reduce gastrointestinal motility and fluid secretion [1].

In the enteropooling test, AQFA significantly reduce the intestinal fluid content at a dose of 800mg/kg body weight. This might be due to inhibition of prostaglandin synthesis. It could also be as a result of increase water and electrolyte re-absorption through the intestinal mucosa [3]. The ability of AQFA to inhibit intestinal fluid accumulation is similar to earlier reports on the antidiarrheal activities of the other plant materials, including root bark of *Cordia africana* and leaves of *Maranta arundinacea* Linn [12,13].

The intestinal motility test using activated charcoal as a marker has been used for over 60 years as a simple and effective method of assessing the effects of laxatives on the gastrointestinal tract [2,14]. This experiment also revealed that AQFA reduced intestinal motility, and this might suggest that *Ficus asperifolia* has the ability to relax intestinal muscles. Many drugs and medicinal herbal extracts that are used in the treatment of diarrhea possess the ability to reduce intestinal contraction and motility [14,15].

Many other plants have also been reported to possess anti-diarrheal activities, such as *Moringa oleifera*, *Albizialebeck*, *Lepidium sativum* [16-18].

Conclusion and Recommendation

This study was carried out to investigate the anti-diarrheal activity of aqueous extract of *Ficus asperifolia* leaf. Base on the beneficial and phytochemical analysis of

Ficus asperifolia in the literature and the results of these experiments we concluded that *Ficus asperifolia* contains pharmacological active substances with significant anti-diarrheal activity.

Studies need to be done on other parts of the plant and different fractions of the extract, to know the major fraction responsible for its anti-diarrheal activity. I also recommend that further studies should use more receptor blockers in order to elucidate the mechanism of its anti-diarrheal activity which exerts antisecretory and antimotility effects.

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